

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Tabin et al.

Serial No: 08/905,572

Filed: August 1, 1997

For: Methods and Compositions for
Regulating Skeletogenic Formation

Art Unit: 1646

Attorney Docket No. HMSU-P07-006

Examiner: C. Kaufman

Commissioner for Patents
Washington, DC 20231

Declaration Under 35 U.S.C. §1.132 of Lee Rubin

Sir:

I, Lee Rubin of Massachusetts, hereby declare as follows:

1. I am an employee of Curis Inc., which licenses the above-described application from Harvard Medical School, the assignee of record.
2. Experiments were performed in collaboration with me, the results of which are depicted in Exhibits A-J, which demonstrate the effects of treating adult tissues with *sonic hedgehog* or *indian hedgehog*. Exhibits A-D summarize the effects of *sonic hedgehog* or *indian hedgehog* on adult bovine cartilage explants grown in 3-dimensional culture in a bioreactor. Exhibit A shows that treatment of these cartilage explants with either *sonic hedgehog* or *indian hedgehog* increases the weight of the explants following four weeks in culture. Exhibit B shows that treatment of adult bovine cartilage explants with either *sonic hedgehog* or *indian hedgehog* increases the cell number in the explants following four weeks in culture. Exhibit C shows that treatment with either *sonic hedgehog* or *indian hedgehog* increases GAG content in explants following four weeks in culture. Exhibit D shows pictures of the cartilage explants following four weeks in culture and demonstrates that explants treated with *sonic hedgehog* resemble and are larger than control explants treated with BMP or IGF. Each of these experiments demonstrates that treatment of adult cartilage tissue with either *sonic hedgehog* or *indian hedgehog* increases the growth and proliferation of the tissue.

Exhibits E-L summarize the effects of systemic delivery of *sonic hedgehog* (either lipid modified or unmodified) to either young mice or adult mice. Exhibits E-F demonstrate that systemic delivery of *sonic hedgehog* to D11 mice induces expression of *patched-1*, as well as

Exhibit 4

expansion of the growth plate. Exhibit G shows the results of BrdU staining and indicates that systemic delivery of *sonic hedgehog* induces chondrocyte proliferation in the growth plate of D11 mice. Exhibits H-L show that, similarly to the results obtained upon systemic administration of *sonic hedgehog* to D11 mice, administration of *sonic hedgehog* to the knee joint of either skeletally mature or aged adult mice results in both the induction of *patched-1* expression and an increase in proliferation. Each of these experiments demonstrate the *in vivo* efficacy of *sonic hedgehog* on cartilage of both young mice and adult mice.

3. The above experiments were performed in accord with the teachings of the abovementioned patent application.

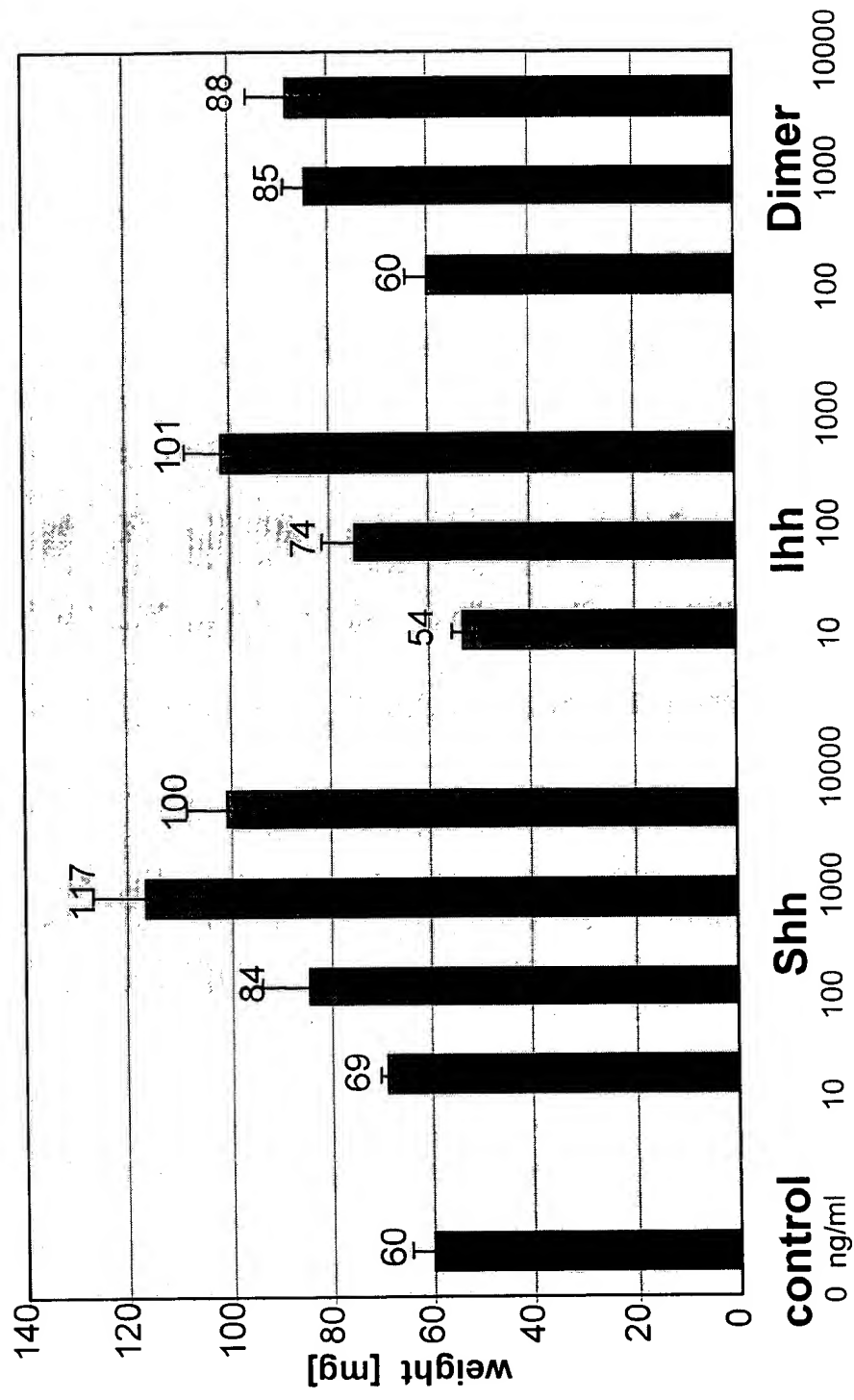
4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Lee Rubin

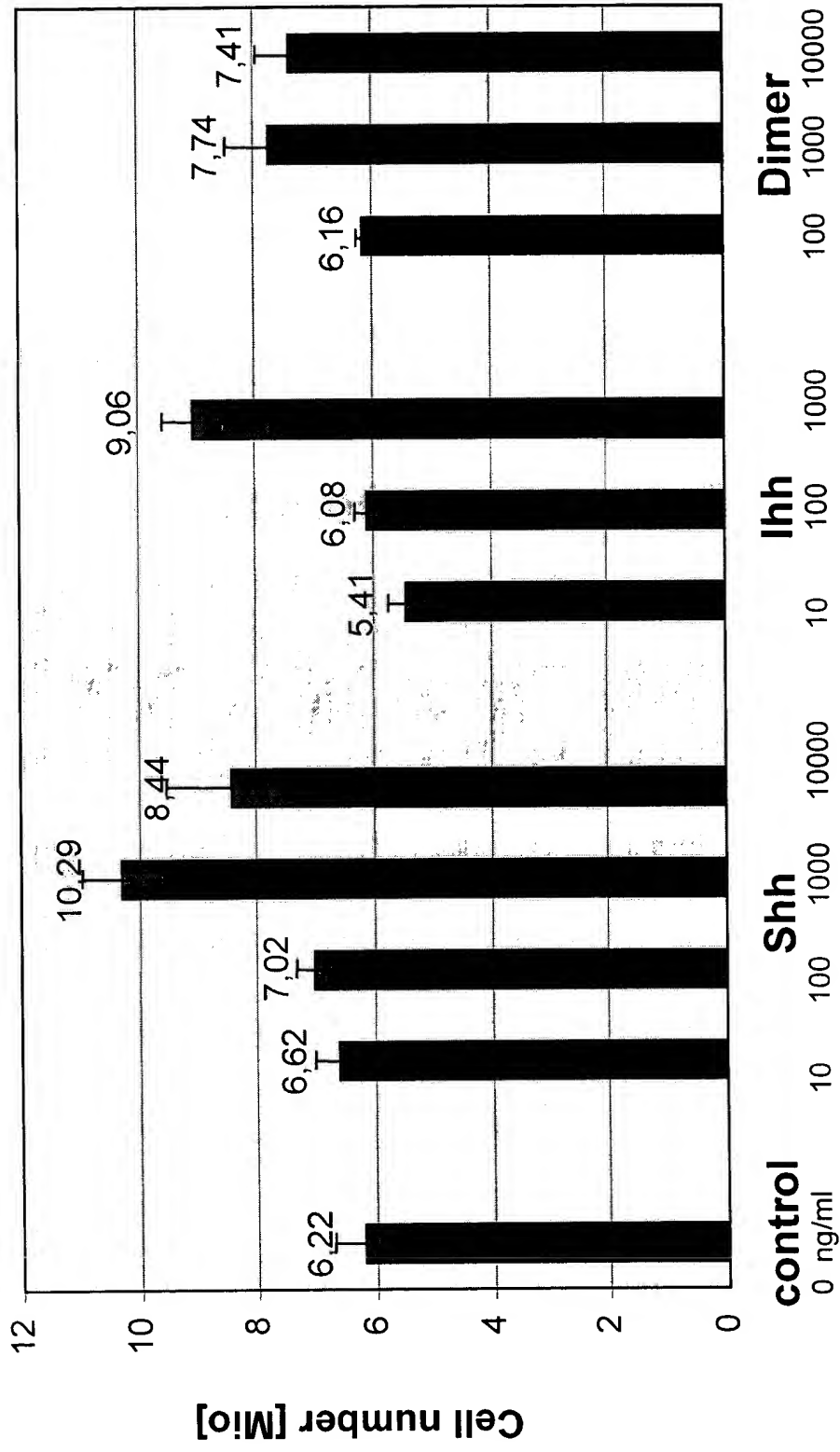
Dated: _____

Signature:

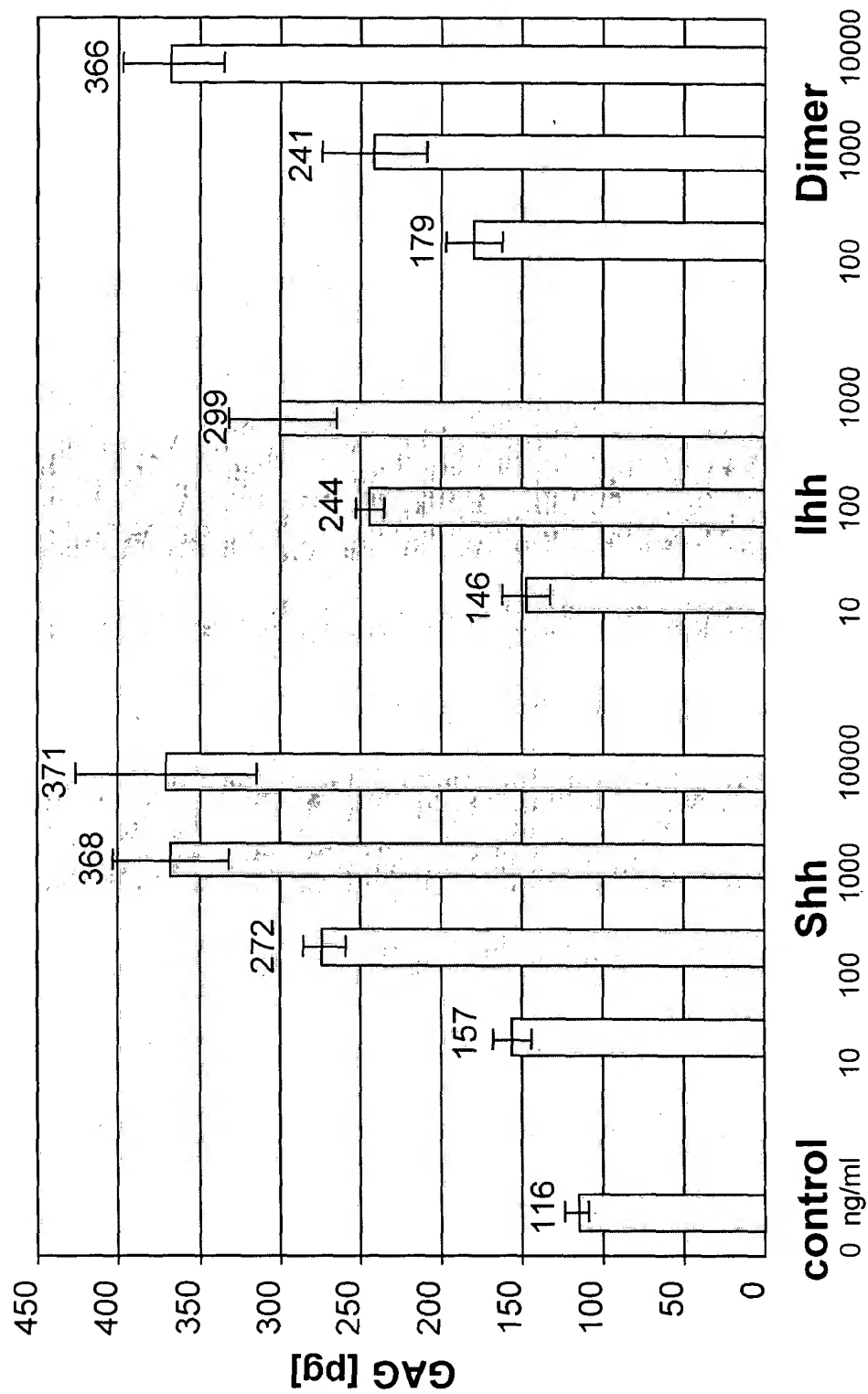
Wet weight / construct (1% serum)



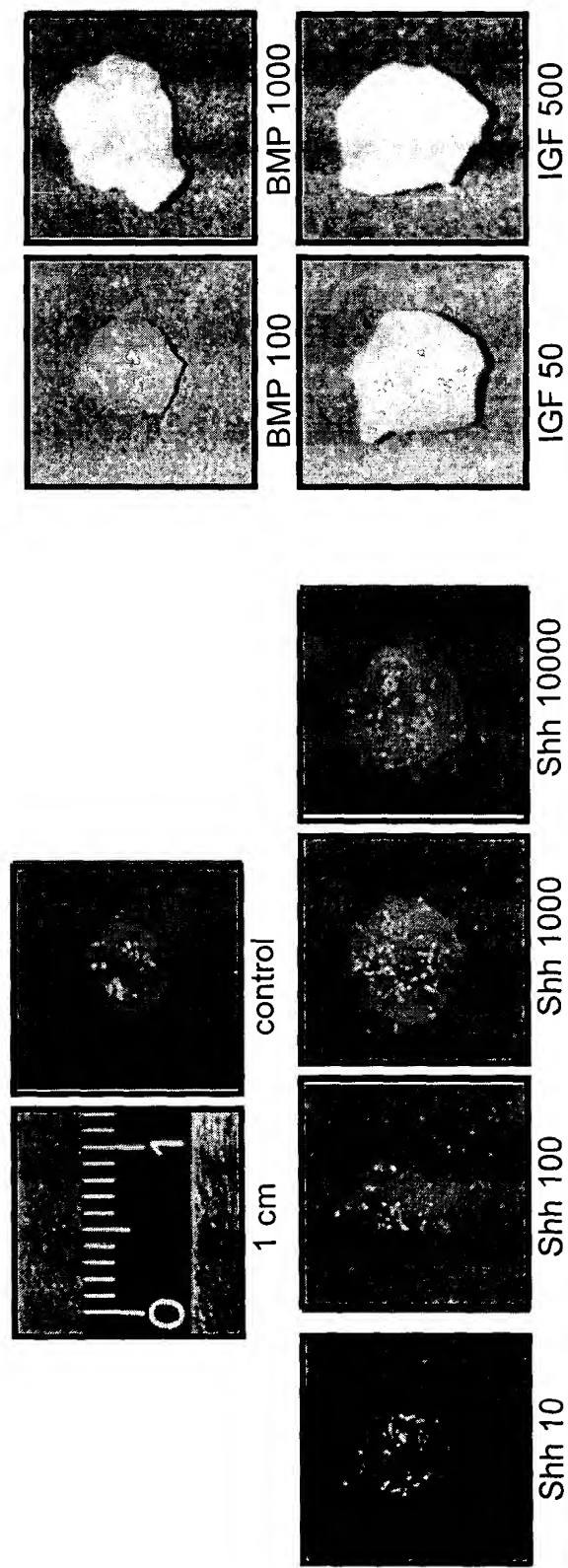
Cell number / dry weight (1% serum)



GAG / cell (1% serum)



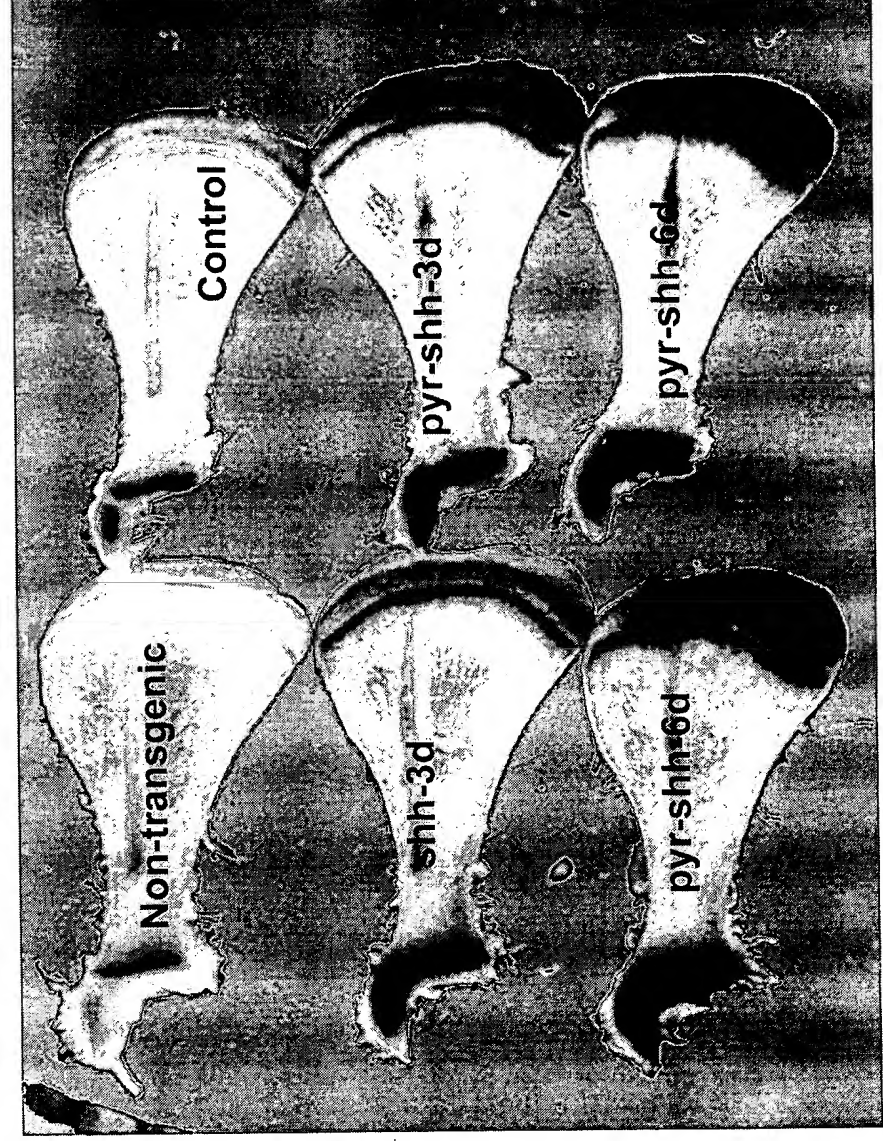
Constructs after 4 weeks of culture (in 1% serum)



In vivo effects of Hh protein

Systemic delivery of Shh in young mice
induces *Ptc-1* expression (D11 mice)
and growth plate expansion

Shh or
pyr-Shh
10 mg/Kg



**Systemic delivery of Shh in young mice
induces growth plate expansion**

**pyr-Shh
10 mg/Kg
(Alcian blue
wholemout)**



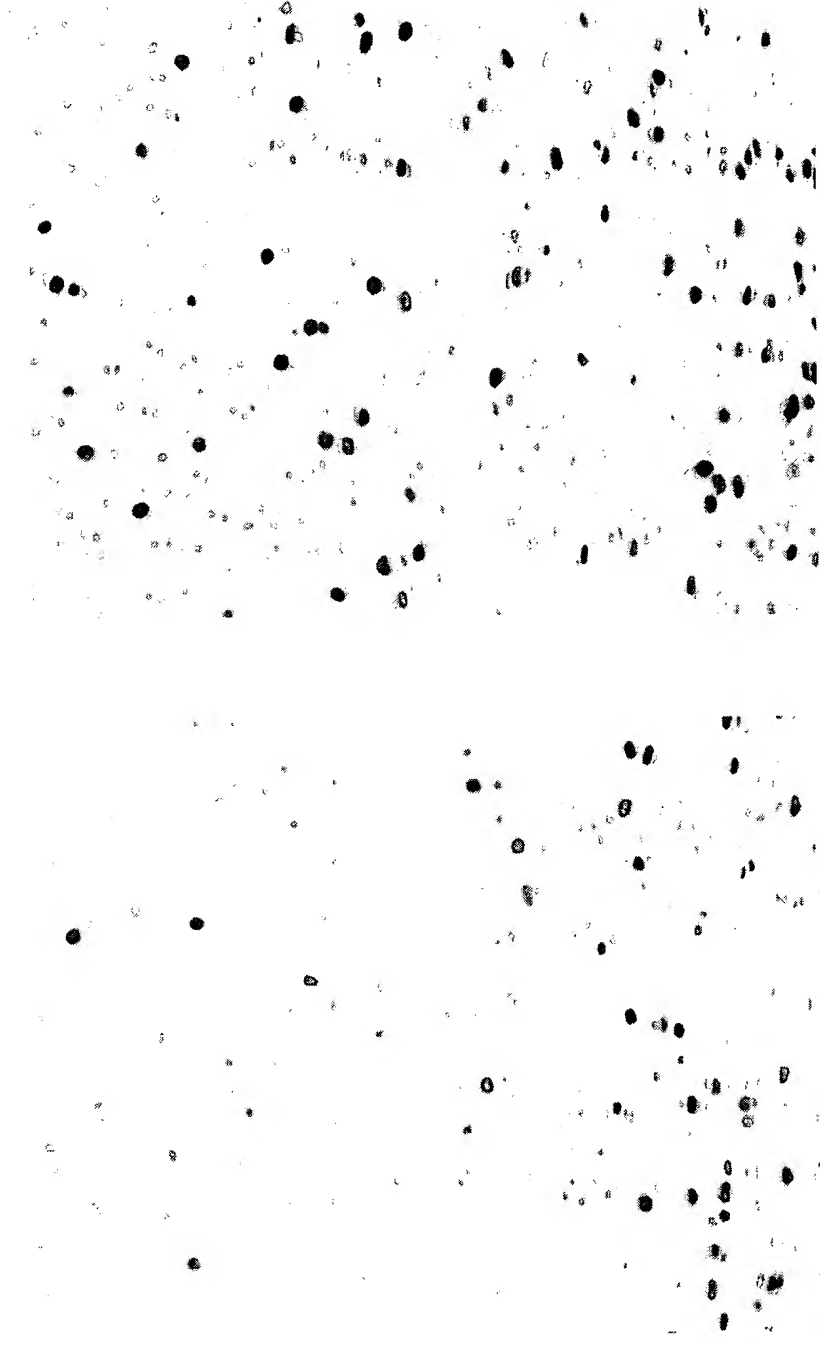
Vehicle



dipal-Shh

**Systemic delivery of octyl-Shh (10 mg/Kg, 2.5 days)
induces chondrocyte proliferation in the
growth plate growing mice**

**BrdU
immunostain**



Vehicle

dipal-Shh

***In vivo* effects of Shh on articular cartilage**

Intra-articular injections of dipal-Shh into the mouse knee joint

Skeletally mature young adults or aged animals (B6 or Ptc-LacZ)

One injection per day, 10 μ l (10 mg or 30 mg)

3-day, 6-day and 11-day treatments

“Recovery” period of 1, 8, 16, or 30 days



Systemic delivery of Hh protein induces *Ptc-1* expression in articular chondrocytes

H&E stain
+
X-gal stain

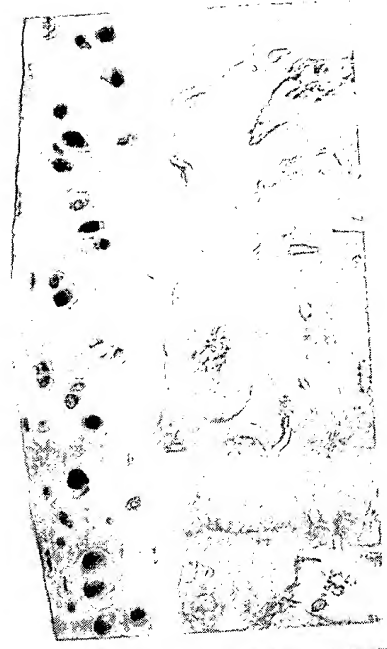
+ vehicle



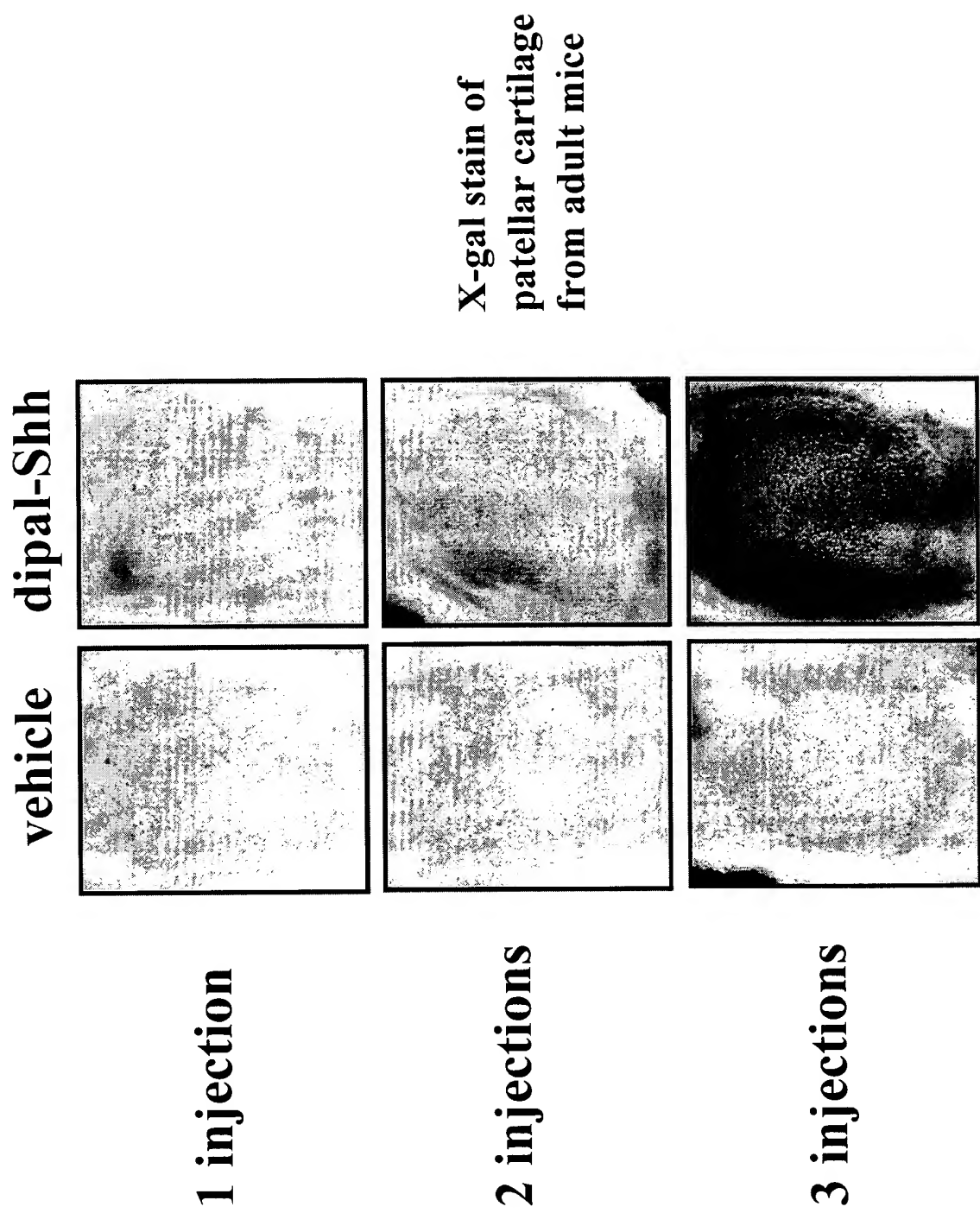
+ dipal-Shh



Adult mouse
femoral head

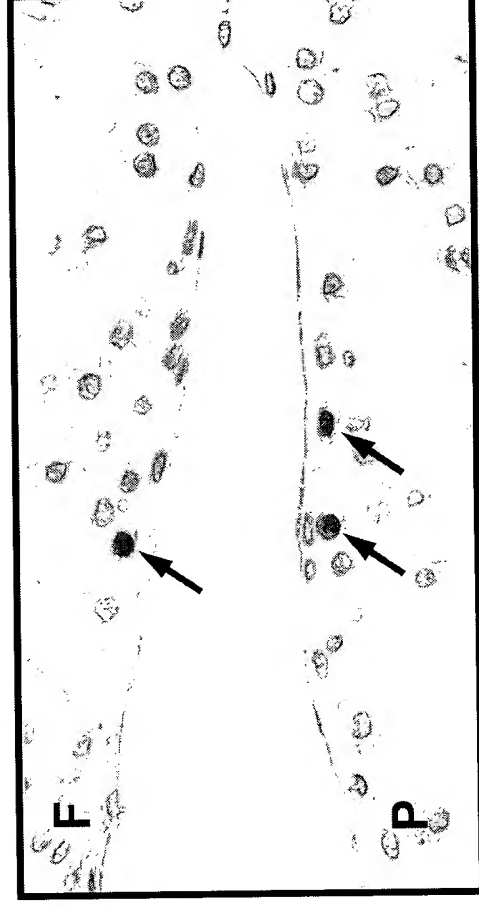


Local delivery of Hh protein induces *Ptc-1* expression in articular chondrocytes



PCNA immunohistochemical assay for chondrocyte proliferation

vehicle



Adult
Patellofemoral
compartment
magnification
= 40X

dipal-Shh
(6 inj.)



dipal-Shh treatment induced chondrocyte proliferation in the patellar cartilage of the mouse knee joint

